Geneaid

Instruction Manual
Ver. 01.20.16
For Research Use Only

Genomic DNA Maxi Kit (Plant)

GPM002 (2 Preparation Sample Kit)
GPM010 (10 Preparation Kit)
GPM025 (25 Preparation Kit)

Advantages

Sample: up to 1 g of fresh plant tissue or up to 250 mg of dry plant tissue
Binding Capacity: 500 µg
Format: genomic DNA maxi columns
Time: within 50 minutes
Elution Volume: 1 ml
Kit Storage: dry at room temperature (15-25°C) for up to 1 year without showing any reduction in performance

Table of Contents

Introduction..... 2
Quality Control..... 2
Kit Components..... 2
Safety Measures..... 3
Quick Protocol Diagram..... 3
Protocol Procedure..... 4
Troubleshooting..... 6
Test Data..... 7
Related Products..... 7

Geneaid Biotech Ltd.
Tel: 886 2 26960999 · Fax: 886 2 26960599 · www.geneaid.com · info@geneaid.com

Introduction

The Genomic DNA Maxi Kit (Plant) provides an efficient method for purifying total DNA (genomic DNA, mitochondrial DNA and chloroplast DNA) from plant tissue and cells. Samples are disrupted by both grinding in liquid nitrogen and lysis buffer incubation. The lysate is treated with RNase A then filtered to remove cell debris and salt precipitates. In the presence of a binding buffer and chaotropic salt, the genomic DNA in the lysate binds to the glass fiber matrix of the spin column. Contaminants are removed using a Wash Buffer (containing ethanol) and the purified genomic DNA is eluted by a low salt Elution Buffer or TE. The procedure does not require DNA phenol extraction or alcohol precipitation, and can be completed in less than 50 mintes. The purified genomic DNA is ready for use in PCR, Real-time PCR, Southern Blotting and RFLP.

Quality Control

The quality of the Genomic DNA Maxi Kit (Plant) is tested on a lot-to-lot basis by isolating genomic DNA from 500 mg young, fresh leaf samples. More than 80 µg of genomic DNA is quantified with a spectrophotometer and analyzed by electrophoresis.

Kit Components

Component	GPM002	GPM010	GPM025
GP1 Buffer	10 ml	50 ml	125 ml
GPX1 Buffer	10 ml	50 ml	125 ml
GP2 Buffer	3 ml	15 ml	30 ml
GP3 Buffer[1] (Add Isopropanol)	8 ml (16 ml)	30 ml (60 ml)	70 ml (140 ml)
W1 Buffer	10 ml	45 ml	130 ml
Wash Buffer[2] (Add Ethanol)	5 ml (20 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer	6 ml	30 ml	60 ml
RNase A (10 mg/ml)	100 µl	550 µl	650 µl x 2
Filter Columns	2	10	25
GD Maxi Columns in Collection Tube	2	10	25
Collection Tube with Cap	2	10	25

[1]Add isopropanol (see the bottle label for volume) to GP3 Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid isopropanol evaporation.

[2]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

Quick Protocol Diagram

Plant sample dissociation

Lysis using either GP1 Buffer or GPX1 Buffer

DNA binding to membrane while contaminants remain suspended

Wash (removal of contaminants while DNA remains bound to membrane)

Elution of pure genomic DNA which is ready for subsequent reactions

Genomic DNA Maxi Kit (Plant) Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.

IMPORTANT BEFORE USE!

1. Add isopropanol (see the bottle label for volume) to GP3 Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid isopropanol evaporation.

2. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

3. Due to various plant species containing different metabolites such as polysaccharides, polyphenols, and proteins, two lysis buffers are provided with this kit. For most common plant species, GP1 buffer ensures purified DNA with high yield and quality. Alternatively, GPX1 Buffer contains detergents which are suitable for plant speices with high a polysaccharide content. Try both lysis buffers to determine the optimal lysis conditions when using a certain plant sample for the first time.

Protocol Procedure

1. Plant Tissue Dissociation

Cut off 0.5 g (up to 1 g) of fresh or frozen plant tissue or 100 mg (up to 250 mg) of dry plant tissue. Homogenize the sample using one of the following methods: **A**. Add liquid nitrogen to a mortar and grind the plant tissue thoroughly using a pestle. Refill the mortar occasionally with liquid nitrogen to ensure the sample remains frozen. Transfer the plant tissue powder to a 15 ml centrifuge tube. **B**. Transfer the sample and 4-5 steel beads (7 mm) to a 15 ml centrifuge tube. Freeze the centrifuge tube with liquid nitrogen, vortex for 20-30 seconds and repeat until the sample becomes a fine powder. Remove the steal beads with a magnet.

2. Lysis

Use GP1 Buffer for lysis of common plant species or GPX1 Buffer for lysis of plants with a high polysaccharide content.

GP1 Buffer: Add **4 ml of GP1 Buffer** and **50 µl of RNase A** into the sample tube and mix by vortex.

NOTE: DO NOT mix GP1 Buffer and RNase A before use.

Incubate at 65ºC for 20 minutes. During incubation, invert the tube every 5 minutes. At this time, preheat the required volume of Elution Buffer (2 ml per sample) to 65ºC (for step 6 DNA Elution). Add 1 ml of GP2 Buffer to the sample lysate, mix by vortex and incubate on ice for 5 minutes. Proceed with step 3 filtration.

GPX1 Buffer: Add **5 ml of GPX1 Buffer** and **50 µl of RNase A** into the sample tube and mix by vortex.

NOTE: DO NOT mix GPX1 Buffer and RNase A before use.

Incubate at 65ºC for 20 minutes. During incubation, invert the tube every 5 minutes. At this time, preheat the required volume of Elution Buffer (2 ml per sample) to 65ºC (for step 6 DNA Elution). Proceed with step 3 filtration.

3. Filtration

Place a **Filter Column** in a 50 ml centrifuge tube. Transfer the sample mixture to the **Filter Column** and centrifuge at 3,000 x g for 5 minutes.

NOTE: Following centrifugation, if some of the sample mixture has not passed through the Filter Column, increase the centrifuge time until all of the sample mixture passes through completely.

Discard the **Filter Column** and carefully transfer the flow-through to a new 50 ml centrifuge tube.

NOTE: If a pellet has formed in the flow-through, carefully transfer the supernatant to a new 50 ml centrifuge tube without disturbing the pellet.

4. DNA Binding

Add a **1.5 volume of GP3 Buffer (make sure isopropanol was added)** to the lysate and vortex immediately for 10 seconds (e.g. add 7.5 ml of GP3 Buffer to 5 ml of lysate).

NOTE: If precipitate appears, break it up as much as possible with a pipette

Transfer the sample mixture (including any precipitate) to the **GD Maxi Column in Collection Tube**. Centrifuge at 3,000 x g for 5 minutes. Discard the flow-through then place the **GD Maxi Column** back in the **Collection Tube**.

5. Wash

Add **4 ml of W1 Buffer** into the **GD Maxi Column** then centrifuge at 3,000 x g for 3 minutes. Discard the flow-through and place the **GD Maxi Column** back in the **Collection Tube**. Add **6 ml of Wash Buffer (make sure ethanol was added)** into the **GD Maxi Column** then centrifuge at 3,000 x g for 3 minutes. Discard the flow-through and place the **GD Maxi Column** back in the **Collection Tube** then centrifuge at 3,000 x g for 10 minutes to dry the column matrix.

Optional Residual Pigment Removal Step

If pigments remain on the column matrix, perform this optional step.

Following Wash Buffer addition, add 4 ml of absolute ethanol into the **GD Maxi Column** then centrifuge at 3,000 x g for 5 minutes. Discard the flow-through then place the **GD Maxi Column** back in the **Collection Tube**. Centrifuge again for 10 minutes at 3,000 x g to dry the column matrix.

6. Elution

Standard elution volume is 1 ml. If less sample is to be used, reduce the elution volume (200-500 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution Step to increase DNA recovery so the total elution volume is approximately 2 ml.

Transfer the dried **GD Maxi Column** to a new **Collection Tube**. Add **1 ml of preheated Elution Buffer[1], TE Buffer[2] or water[3]** into the center of the column matrix. Let stand for 5 minutes or until the Elution Buffer, TE Buffer or water is absorbed by the matrix. Centrifuge at 3,000 x g for 3 minutes to elute purified DNA.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the GD Maxi Column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GD Maxi Column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the GD Maxi Column matrix and is completely absorbed. DNA eluted in water should be stored at -20ºC to avoid degradation.

Troubleshooting

Low Yield

Incomplete buffer preparation

1. Add isopropanol (see the bottle label for volume) to GP3 Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid isopropanol evaporation.

2. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Clogged Column

Too much sample was used. Reduce the sample volume or separate it into multiple tubes.

Precipitate was formed at DNA Binding Step

Reduce the sample material. Prior to loading the column, break up precipitate in the ethanol-added lysate.

Incorrect DNA elution step.

Ensure that Elution Buffer, TE or water is added into the **CENTER** of the GD Maxi Column matrix and is completely absorbed. Use pre-heated Elution Buffer, TE, or water (60~70ºC). If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Repeating the elution step will increase yield. Repeating the elution step using the eluate only will increase DNA concentration.

Eluted DNA Does Not Perform Well In Downstream Applications

Residual ethanol contamination.
Following the wash step, dry the GD Maxi Column with additional centrifugation at 3,000 x g for 5 minutes to ensure the membrane is completely dry.

Genomic DNA Maxi Kit (Plant) Functional Test Data

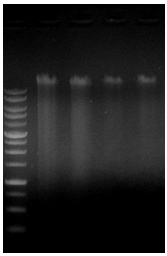

Figure 1. Genomic DNA (approximately 30 kb) was extracted using the Genomic DNA Maxi Kit (Plant). 0.5 g of fresh lemon leaves and 0.5 g of fresh coffee leaves were ground into a fine powder followed by DNA extraction. 2 µl aliquots of purified genomic DNA from 1 ml eluates were analyzed by electrophoresis on a 0.8% agarose gel. M = Geneaid 1 Kb DNA Ladder

Sample	DNA Conc.	260/280	Yield
Lemon Leaf	80.6 ng/µl	1.81	80.6 µg
Lemon Leaf	82.2 ng/µl	1.80	82.2 µg
Coffee Leaf	37.3 ng/µl	1.79	37.3 µg
Coffee Leaf	36.4 ng/µl	1.75	36.4 µg

Related DNA Extraction Products

Genomic DNA Extraction		
Product	Package Size	Catalogue Number
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	GB100/300
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	GDM010/25
Genomic DNA Mini Kit (Tissue)	50/100/300 preps	GT050/100/300
gSYNC™ DNA Extraction Kit	50/100/300 preps	GS050/100/300
Genomic DNA Mini Kit (Plant)	100 preps	GP100
Genomic DNA Maxi Kit (Plant)	10/25 preps	GPM10/25
GENEzol™ DNA Reagent Plant	100/200 rxns	GR100/200
Presto™ Mini gDNA Yeast Kit	100/300 preps	GBY100/300
Presto™ Mini gDNA Bacteria Kit	100/300 preps	GBB100/101/300/301
Geneius™ Micro DNA Extraction Kit	100/300 preps	GMB100/300
Presto™ Buccal Swab gDNA Extraction Kit	100/300 preps	GSK100/300
Presto™ 96 Well Blood Genomic DNA Extraction Kit	4/10 x 96 preps	96GBP04/10
Presto™ 96 Well Plant Genomic DNA Extraction Kit	4/10 x 96 preps	96GPP04/10

For additional product information please visit www.geneaid.com. Thank you!

Geneaid

INTERNATIONAL

UKAS QUALITY MANAGEMENT

Registration Number 046

CERTIFICATE NO. QAIC/TW/50077

www.geneaid.com